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(71) Applicant: Ajinomoto Co., Inc.
Tokyo 104 (JP)

(72) Inventors:
- Matsuzaki, Yumi,
c/o Ajinimoto Co., Inc.
Kawasaki-shi, Kanagawa (JP)
- Kimura, Eiichiro,
c/o Ajinimoto Co., Inc.
Kawasaki-shi, Kanagawa (JP)
- Nakamatsu, Tsuyoshi,
c/o Ajinimoto Co., Inc.
Kawasaki-shi, Kanagawa (JP)
- Kurahashi, Osamu,
c/o Ajinimoto Co., Inc.
Kawasaki-shi, Kanagawa (JP)
- Kawahara, Yoshio,
c/o Ajinimoto Co., Inc.
Kawasaki-shi, Kanagawa (JP)
- Sugimoto, Shinichi,
c/o Ajinimoto Co., Inc.
Kawasaki-shi, Kanagawa (JP)

(74) Representative: HOFFMANN - EITLE
Patent- und Rechtsanwälte
Arabellastrasse 4
81925 München (DE)

(54) Plasmid capable of autonomous replication in coryneform bacteria

(57) A plasmid isolable from *Corynebacterium thermoaminogenes*, which comprises a gene coding for a Rep protein having the amino acid sequence shown in SEQ ID NO: 2 or an amino acid sequence having homology of 90% or more to the amino acid sequence shown in SEQ ID NO: 2, and has a size of about 4.4 kb or about 6 kb, or a derivative thereof.

EP 1 076 094 A2

Description

BACKGROUND OF THE INVENTION

[0001] The present invention relates to a novel plasmid derived from *Corynebacterium thermoaminogenes*. The plasmid of the present invention can be utilized for improving of coryneform bacteria, which are used as bacteria for producing useful substances such as L-amino acids.

[0002] Amino acids including L-glutamic acid and L-lysine are produced by fermentative methods using the so-called coryneform bacteria, which generally belong to the genus *Brevibacterium, Corynebacterium* or *Microbacterium*, or variant strains thereof (Amino Acid Fermentation, Gakkai Shuppan Center, pp.195-215, 1986).

[0003] In the industrial fermentative production of amino acids, besides improvement in yield relative to saccharides, shortening of culture time, improvement in amino acid accumulation concentration and so forth, use of an elevated culture temperature is considered important as a technical factor that raises economical efficiency. That is, culture is usually performed at optimum fermentation temperature, and the optimum temperature is 31.5°C for *Corynebacterium glutamicum*. After the culture is started, heat is generated during the fermentation, and hence amino acid production is markedly reduced if this heat output is not removed. Therefore, cooling equipment is required in order to maintain the temperature of the culture broth to be optimum. On the other hand, if the culture temperature can be elevated, it becomes possible to decrease energy required for cooling and the cooling equipment can be made small.

[0004] Among coryneform bacteria, *Corynebacterium thermoaminogenes* has been isolated as a coryneform bacterium that can grow in a high temperature region (Japanese Patent Application Laid-open (Kokai) No. 63-240779). Whereas growth of *Corynebacterium glutamicum* is markedly suppressed at 40°C, *Corynebacterium* thermoaminogenes can grow at a temperature of about 40°C or higher, and is considered to be suitable for high temperature fermentation.

[0005] Currently, improving relying on DNA recombination techniques is progressing in *Escherichia coli* or coryneform bacteria. In order to improve microorganisms by DNA recombination techniques, even plasmids derived from microorganisms belonging to another species or genus or broad host spectrum vectors are often used. However, plasmids proper to objective microorganisms of improving are generally used. In particular, when optimum culture temperature for the objective microorganism of the improving is different from that of microorganisms of the same species or genus, it is preferable to use a plasmid proper to the microorganism.

[0006] So far obtained as plasmids derived from coryneform bacteria are pAM330 from *Brevibacterium lactofermentum* ATCC13869 (Japanese Patent Application Laid-open (Kokai) No. 58-67669), pBL1 from *Brevibacterium lactofermentum* ATCC21798 (Santamaria. R. et al., J. Gen. Microbiol., 130, pp.2237-2246, 1984), pHM1519 from *Corynebacterium glutamicum* ATCC13058 (Japanese Patent Application Laid-open (Kokai) No. 58-77895), pCG1 from *Corynebacterium glutamicum* ATCC31808 (Japanese Patent Application Laid-open (Kokai) No. 57-134500) and pGA1 from *Corynebacterium glutamicum* DSM58 (Japanese Patent Application Laid-open (Kokai) No. 9-2603011).

[0007] However, no plasmid proper to *Corynebacterium thermoaminogenes* has obtained at present.

SUMMARY OF THE INVENTION

[0008] An object of the present invention is to provide a plasmid useful for improving of the coryneform bacterium that can grow at an elevated temperature, *Corynebacterium thermoaminogenes*.

[0009] The inventors of the present invention found that *Corynebacterium thermoaminogenes* AJ12340 (FERM BP-1539), AJ12308 (FERM BP-1540), AJ12309 (FERM BP-1541) and AJ12310 (FERM BP-1542) each harbored a cryptic plasmid proper to each strain, and successfully isolated and identified each plasmid. Thus, they accomplished the present invention.

[0010] That is, the present invention provides a plasmid isolable from *Corynebacterium thermoaminogenes*, which comprises a gene (*rep* gene) coding for a Rep protein having the amino acid sequence shown in SEQ ID NO: 2 or an amino acid sequence having homology of 90% or more to the foregoing amino acid sequence, and has a size of about 4.4 kb or about 6 kb, or a derivative thereof.

[0011] Examples of the aforementioned plasmid include a plasmid isolable from *Corynebacterium thermoaminogenes* AJ12340 (FERM BP-1539), AJ12308 (FERM BP-1540) or AJ12310 (FERM BP-1542), which has a size of about 4.4 kb and is represented by the restriction map shown in Fig. 1, and a plasmid isolable from *Corynebacterium thermoaminogenes* AJ12309 (FERM BP-1541), which has a size of about 6 kb and is represented by the restriction map shown in Fig. 2.

[0012] Specific examples of the aforementioned plasmid include a plasmid which comprises a gene coding for a Rep protein having the amino acid sequence shown in SEQ ID NO: 2, 4 or 6, and a plasmid which comprises a gene coding for a Rep protein having the amino acid sequence shown in SEQ ID NO: 8.

BRIEF EXPLANATION OF THE DRAWINGS

[0013]

Fig. 1 is a restriction map of the plasmids pYM1, pYM2 and pYM3 of the present invention.
Fig. 2 is a restriction map of the plasmid pYM4 of the present invention.
Fig. 3 shows construction of pYMFK.
Fig. 4 shows construction of pYMK.
Fig. 5 shows construction of pYMC.
Fig. 6 shows construction of pK1.

DETAILED DESCRIPTION OF THE INVENTION

[0014] The plasmid of the present invention can be isolated form *Corynebacterium thermoaminogenes* AJ12340 (FERM BP-1539), AJ12308 (FERM BP-1540), AJ12309 (FERM BP-1541) or AJ12310 (FERM BP-1542) according to a usual method for preparing a plasmid such as the alkali method (Text for Bioengineering Experiments, Edited by the Society for Bioscience and Bioengineering, Japan, p.105, Baifukan, 1992). As for FERM BP-1539, its original deposition, which was deposited at the National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology (postal code 305-8566, 1-3 Higashi 1-chome, Tsukuba-shi, Ibaraki-ken, Japan) on March 13, 1987 and given an accession number of FERM P-9277, was transferred to an international deposition under the provisions of the Budapest Treaty on October 27, 1987 and has been deposited at the same depository. As for FERM BP-1540, FERM BP-1541 and FERM BP-1542, their original depositions, which were deposited at the aforementioned depository on March 10, 1987 and given accession numbers of FERM P-9244, FERM P-9245 and FERM P-9246, were transferred to international depositions under the provisions of the Budapest Treaty on October 27, 1987 and have been deposited at the same depository.

[0015] The Inventors of the present invention isolated and identified plasmids each proper to each of the aforementioned *Corynebacterium thermoaminogenes* AJ12308 (FERM BP-1540), AJ12310 (FERM BP-1542), AJ12340 (FERM BP-1539) and AJ12309 (FERM BP-1541) from them, and designated as pYM1, pYM2, pYM3 and pYM4 in that order. These plasmids are plasmids that exist as double-stranded circular DNA in a cell of *Corynebacterium thermoaminogenes*. The nucleotide sequence of the *rep* gene contained in pYM1 is shown in SEQ ID NO: 1, the nucleotide sequence of the rep gene contained in pYM2 is shown in SEQ ID NO: 3, the nucleotide sequence of the rep gene contained in pYM3 is shown in SEQ ID NO: 5, and the nucleotide sequence of the *rep* gene contained in pYM4 is shown in SEQ ID NO: 7. The amino acid sequences that can be encoded by the *rep* genes contained in these plasmids are shown in SEQ ID NOS: 2, 4, 6 and 8. pYM1, pYM2 and pYM3 each have a size of about 4.4 kb. pYM4 has a size of about 6 kb.

[0016] Numbers and sizes of fragments that can be obtained when pYM1, pYM2 and pYM3 are digested with typical restriction enzymes are shown in Table 1. Numbers and sizes of fragments that can be obtained when pYM4 is digested with typical restriction enzymes are shown in Table 2. Further, a restriction map of pYM1, pYM2 and pYM3 is shown in Fig. 1, and a restriction map of pYM4 is shown in Fig. 2.

Table 1

Restriction enzyme	Number of digestion site	DNA fragment (kb)
*Bgl*II	0	-
*Bam*HI	2	1.8, 2.6
*Bst*PI	1	4.4
*Eco*RI	1	4.4
*Hinc*II	4	0.3, 0.5, 2.0, 1.6
*Hind*III	0	-
*Kpn*I	0	-
*Nae*I	2	0.1, 4.3
*Nco*I	1	4.4
*Nhe*I	2	1.8, 2.6

Table 1 (continued)

Restriction enzyme	Number of digestion site	DNA fragment (kb)
*Pma*CI	1	4.4
*Sac*I	0	-
*Sal*I	0	-
*Sac*II	3	0.1, 1.4, 2.9
*Sma*I	3	0.1, 1.8, 2.5
*Sph*I	0	-
*Tth*111I	1	4.4
*Xba*I	0	-

Table 2

Restriction enzyme	Number of digestion site	DNA fragment (kb)
*Bgl*II	1	6.0
*Bam*HI	2	3.8, 2.2
*Bst*PI	2	1.2, 4.8
*Eco*RI	1	6.0
*Hinc*II	4	0.3, 0.4, 1.2, 1.7, 2.4
*Hind*III	0	-
*Kpn*I	0	-
*Nae*I	2	0.1, 5.9
*Nco*I	3	0.2, 2.8, 3.0
*Nhe*I	3	0.1, 2.3, 3.6
*Pma*CI	0	-
*Sac*I	0	-
*Sal*I	0	-
*Sac*II	5	0.1, 0.2, 0.9, 1.8, 3.0
*Sma*I	2	0.1, 5.9
*Sph*I	0	-
*Tth*111I	0	-
*Xba*I	0	-

[0017] Determination of the nucleotide sequence of the plasmid of the present invention revealed that pYM1, pYM2, and pYM3 contained 4368 bp, 4369 bp and 4369 bp, respectively, and they had substantially the same structure and showed homology of 99.9% to one another on the nucleotide sequence level. Further, pYM4 contained 5967 bp and it showed extremely high homology to pYM1, pYM2 and pYM3 for the region of about 4.4 kb except for the region of about 1.6 kb, while it showed homology of about 81% to them as a whole.

[0018] The plasmids contain respective *rep* genes which show high homology to one another. Homology was compared for the amino acid sequences of the Rep proteins encoded by the *rep* genes (SEQ ID NOS: 2, 4, 6 and 8) and the amino acid sequences of the Rep proteins encoded by rep genes of known plasmids derived from coryneform bacteria. Homology of 99% or more was observed among pYM1, pYM2 and pYM3, and homology of 81.91% was observed

between pYM2 and pYM4. On the other hand, they showed no homology to the known plasmid pAM330 of a coryneform bacterium, and they showed homology of 80% or less to pGA1 and pCG1. The results are shown in Table 3. Thus, the plasmid of the present invention and the known plasmids of coryneform bacteria are distinguishable based on the homology of the Rep protein.

[0019] The homology is calculated according to the method described in Takashi, K. and Gotoh, O., J. Biochem., 92, 1173-1177 (1984).

Table 3

Homology of amino acid sequences of Rep protein encoded by various plasmids				
	PYM2	pYM4	pGA1	pCG1
PYM2	-	81.91%	68.01%	70.73%
PYM4	-	-	69.39%	70.23%
PGA1	-	-	-	75.31%
PCG1	-	-	-	-

[0020] Since the plasmid of the present invention can sufficiently replicate in cells of coryneform bacteria including *Corynebacterium thermoaminogenes*, genetic information of a foreign gene can be expressed in a host microorganism by inserting the foreign gene at any site of the plasmid or the derivative thereof, and transforming the host microorganism with the obtained recombinant plasmid.

[0021] Examples of coryneform bacteria are listed below.

Corynebacterium acetoacidophilum
Corynebacterium acetoglutamicum
Corynebacterium callunae
Corynebacterium glutamicum
Corynebacterium thermoaminogenes
Corynebacterium lilium (Corynebacterium glutamicum)
Corynebacterium melassecola
Brevibacterium divaricatum (Corynebacterium glutamicum)
Brevibacterium lactofermentum (Corynebacterium glutamicum)
Brevibacterium saccharolyticum
Brevibacterium immariophilum
Brevibacterium roseum
Brevibacterium flavum (Corynebacterium glutamicum)
Brevibacterium thiogenitalis

[0022] A derivative of the plasmid of the present invention means a plasmid composed of a part of the plasmid of the present invention, or a part of the plasmid of the present invention or the plasmid of present invention and another DNA sequence. The part means a part containing a region essential for the autonomous replication of the plasmid. The plasmid of the present invention can replicate in a host microorganism even if a region other than the region essential for the autonomous replication of the plasmid (replication control region), that is, the region other than the region containing the replication origin and genes necessary for the replication, is deleted. In addition, a plasmid including such a deletion has a smaller size. Therefore, a plasmid having such a deletion is preferred for use as a vector. Further, if a marker gene such as a drug resistance gene is inserted into the plasmid of the present invention or a part thereof, it becomes easy to detect transformants thanks to phenotype of the marker gene in the transformants. Examples of such a marker gene that can be used in the host include a chloramphenicol resistance gene, kanamycin resistance gene, streptomycin resistance gene, tetracycline resistence gene, trimethoprim resistance gene, erythromycin resistance gene and so forth.

[0023] Further, if the plasmid of the present invention is made as a shuttle vector autonomously replicable in coryneform bacteria and other bacteria such as *Escherichia coli* by ligating the plasmid of the present invention or a part thereof with a plasmid autonomously replicable in the other bacteria such as *Escherichia coli* or a part thereof containing a replication control region thereof, manipulations such as preparation of plasmid and preparation of recombinant plasmid containing a target gene can be performed using *Escherichia coli*. Examples of the plasmid

autonomously replicable in *Escherichia coli* include, for example, pUC19, pUC18, pBR322, pHSG299, pHSG298, pHSG399, pHSG398, RSF1010, pMW119, pMW118, pMW219, pMW218 and so forth.

[0024] Although pYM1, pYM2, pYM3 and pYM4 themselves are characterized by the restriction maps shown in Figs. 1 and 2, the plasmid of present invention is not necessarily required to have these restriction maps, and any restriction site may be deleted so long as such deletion does not affect the autonomous replication ability. Further, the plasmid of the present invention may contain a restriction site that is not contained in pYM1, pYM2, pYM3 and pYM4.

[0025] The derivative of the plasmid as described above can be constructed in the same manner as the conventionally known construction of cloning vectors, expression vectors and so forth. In order to construct the derivative, it is preferable to determine the nucleotide sequences of pYM1, pYM2, pYM3 and pYM4. The nucleotide sequence can be determined by known methods such as the dideoxy method.

[0026] In order to insert a foreign gene into the plasmid or the derivative thereof of the present invention, it is convenient to insert it into a restriction site of the plasmid or the derivative thereof. As such a restriction site, one present as a single digestion site is preferred. In order to insert a foreign gene, the plasmid and a source of the foreign gene such as genome DNA can be partially or fully digested with one or more restriction enzymes that provide the same cohesive ends for the both, e.g., the same restriction enzyme, and they can be ligated under a suitable condition. They may also be ligated at blunt ends.

[0027] For preparation of plasmid DNA, digestion and ligation of DNA, transformation and so forth, those methods well known to those skilled in the art may be employed. Such methods are described in Sambrook, J., Fritsch, E.F., and Maniatis, T., "Molecular Cloning: A Laboratory Manual, Second Edition", Cold Spring Harbor Laboratory Press (1989) and so forth.

[0028] According to the present invention, a novel plasmid derived from *Corynebacterium thermoaminogenes* is provided as described above.

EXAMPLES

[0029] Hereafter, the present invention will be explained in more detail with reference to the following examples.

Example 1

Isolation and characterization of plasmids from *Corynebacterium thermoaminogenes* (FERM BP-1539, FERM BP-1540, FERM BP-1541, FERM BP-1542)

[0030] *Corynebacterium thermoaminogenes* AJ12340 (FERM BP-1539), AJ12308 (FERM BP-1540), AJ12309 (FERM BP-1541) and AJ12310 (FERM BP-1542) were cultured for 12 hours in CM2B liquid medium (Bacto-trypton (Difco): 1%, Bacto-yeast-extract (Difco): 1%, NaCl: 0.5%, biotin: 10 μg/L), and plasmid DNA fractions were obtained by the alkali method (Text for Bioengineering Experiments, Edited by the Society for Bioscience and Bioengineering, Japan, p.105, Baifukan, 1992). When these fractions were analyzed by agarose gel electrophoresis (Sambrook, J., Fritsch, E.F., and Maniatis, T., "Molecular Cloning: A Laboratory Manual, Second Edition", Cold Spring Harbor Laboratory Press (1989)), DNA bands were detected for all of the cases, and hence it was demonstrated that the aforementioned strains harbored plasmids. The plasmids prepared from FERM BP-1540, FERM BP-1542 and FERM BP-1539 were designated as pYM1, pYM2 and pYM3, respectively. The plasmid prepared from FERM BP-1541 was designated as pYM4. The plasmids pYM1, pYM2 and pYM3 each had a length of about 4.4 kb, and the plasmid pYM4 had a length of about 6.0 kb.

[0031] The plasmids pYM1, pYM2, pYM3 and pYM4 were digested with restriction enzymes *Bgl*II, *Bam*HI, *Bst*PI, *Eco*RI, *Hin*cII, *Hin*dIII, *Kpn*I, *Nae*I, *Nco*I, *Nhe*I, *Pma*CI, *Sac*I, *Sac*II, *Sal*I, *Sma*I, *Sph*I, *Tth*111I and *Xba*I (produced by Takara Co.), and lengths of the produced DNA fragments were measured by agarose gel electrophoresis. The electrophoresis was performed at 100 V/cm and a constant voltage for several hours by using 0.8% agarose gel. As molecular weight markers, λ phage DNA (Takara Shuzo) digested with a restriction enzyme *Hin*dIII was used. The results obtained for pYM1, pYM2 and pYM3 are shown in Table 1. The results obtained for pYM4 are shown in Table 2. The restriction map of pYM1, pYM2 and pYM3 is shown in Fig. 1, and the restriction map of pYM4 is shown in Fig. 2, which were prepared based on the above results.

[0032] The results of nucleotide sequencing of pYM1, pYM2, pYM3 and pYM4 by the dideoxy method are shown in SEQ ID NOS: 1, 3, 5 and 7 in that order.

Example 2

Construction of shuttle vector pYMFK containing Km resistance gene derived from *Streptococcus faecalis*

[0033] As a region necessary for efficient replication of pYM2 in coryneform bacteria, there are present an AT-rich region upstream from *rep* and a region affecting copy number downstream from *rep*, besides the region coding for *rep*.

[0034] Therefore, in order to obtain a shuttle vector that can replicate in coryneform bacteria and *E. coli* without impairing the replication ability of pYM2, a region enabling autonomous replication in *E. coli* and a selection marker were inserted into sites in the vicinity of the *Bst*PI site of pYM2.

[0035] First, a vector having a drug resistance gene of *S. faecalis* was constructed. The kanamycin resistance gene of *S. faecalis* was amplified by PCR from a known plasmid containing that gene. The nucleotide sequence of the kanamycin resistance gene of the *S. faecalis* has already been elucidated (Trieu-Cuot, P. and Courvalin, P., *Gene*, 23 (3), pp.331-341 (1983)). Based on this sequence, the primers having the nucleotide sequences shown as SEQ ID NOS: 16 and 17 were synthesized, and PCR was performed by using pDG783 (Anne-Marie Guerout-Fleury et al., *Gene, 167*, pp.335-337 (1995)) as a template to amplify a DNA fragment containing the kanamycin resistance gene and its promoter.

[0036] The above DNA fragment was purified by using SUPREC02 produced by Takara Shuzo Co., Ltd., completely digested with restriction enzymes *Hind*III and *Hinc*II, and blunt-ended. The blunt-ending was performed by using Blunting Kit produced by Takara Shuzo Co., Ltd. This DNA fragment and an amplification product obtained by PCR utilizing the primers having the nucleotide sequences shown as SEQ ID NOS: 18 and 19 and pHSG399 (see S. Takeshita *et al.*, *Gene*, *61*, pp.63-74 (1987)) as a template and purification and blunt-ending of the PCR product were mixed and ligated. The ligation reaction was performed by using DNA Ligation Kit ver.2 produced by Takara Shuzo Co., Ltd. Competent cells of *Escherichia coli* JM109 (produced by Takara Shuzo Co., Ltd.) were transformed with the ligated DNA, and applied to L medium (10 g/L of Bacto trypton, 5 g/L of Bacto yeast extract, 5 g/L of NaCl, and 15 g/L of agar, pH 7.2) containing 10 μg/ml of IPTG (isopropyl-β-D-thiogalactopyranoside), 40 μg/ml of X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactoside) and 25 μg/ml of kanamycin, and cultured overnight. Then, the formed blue colonies were picked up, and subjected to single colony isolation to obtain transformants.

[0037] Plasmids were prepared from the transformants by using the alkaline method (Text for Bioengineering Experiments, Edited by the Society for Bioscience and Bioengineering, Japan, p.105, Baifukan, 1992), and restriction maps were prepared. A plasmid having a restriction map equivalent to that shown at a lower position in Fig. 6 was designated as pK1. This plasmid is stably harbored in *Escherichia coli*, and imparts kanamycin resistance to a host. Moreover, since it contains the *lacZ'* gene, it is suitable for use as a cloning vector.

[0038] Then, a region containing the replication origin was amplified by Pyrobest-Taq (Takara Shuzo Co., Ltd.) using pYM2 extracted from *C. thermoaminogenes* AJ12310 (FERM BP-1542) as a template (The entire nucleotide sequence of pYM2 is shown in SEQ ID NO: 9.) and the following primers prepared based on a sequence in pYM2 near the *Bst*PI site:

S1: 5'-AAC CAG GGG GAG GGC GCG AGG C-3' (SEQ ID NO: 10)
S3: 5'-TCT CGT AGG CTG CAT CCG AGG CGG GG-3' (SEQ ID NO: 11)

The reaction condition was 94°C for 5 minutes, then a cycle of 98°C for 20 seconds and 68°C for 4 minutes, which was repeated for 30 cycles, and 72°C for 4 minutes. After the reaction, the mixture was stored at 4°C.

[0039] The obtained amplified fragment was purified by using MicroSpin TM S-400 HR columns produced by Amersham Pharmacia Biotech Co., blunt-ended by using DNA Blunting Kit produced by Takara Shuzo Co., Ltd., and then ligated to pK1 treated with *Hinc*II by using DNA Ligation Kit. ver. 2 produced by Takara Shuzo Co., Ltd. Competent cells of *Escherichia coli* JM109 (produced by Takara Shuzo) were transformed with the ligated DNA to obtain transformant strains.

[0040] Plasmids were prepared from the transformant strains using the alkali method (Text for Bioengineering Experiments, Edited by the Society for Bioscience and Bioengineering, Japan, p.105, Baifukan, 1992) and restriction maps of the plasmids were prepared. One showing a restriction map equivalent to that shown at a lower position in Fig. 3 was designated as pYMFK. pYMFK had a size of about 7.0 kb, and was able to autonomously replicate in *E. coli* and coryneform bacteria and impart Km resistance to a host.

Example 3

Construction of pYMK containing Km resistance gene derived from Tn903

[0041] A region containing the replication origin was amplified in the same manner as in Example 2 by using pYM2

extracted from *C. thermoaminogenes* AJ12310 (FERM BP-1542) as a template and the following primers:

S1XbaI: 5'-GCT CTA GAG CAA CCA GGG GGA GGG CGC GAG GC-3' (SEQ ID NO: 12)
S3XbaI: 5'-GCT CTA GAG CTC TCG TAG GCT GCA TCG GAG GCG GGG-3' (SEQ ID NO: 13)

[0042] The obtained amplified fragment was purified by using MicroSpin TM S-400 HR columns produced by Amersham Pharmacia Biotech Co., digested with a restriction enzyme *XbaI* produced by Takara Shuzo Co., Ltd., and then ligated to a fragment obtained by fully digesting pHSG299 (Takara Shuzo Co., Ltd.) with *XbaI* by using DNA Ligation Kit. ver. 2 produced by Takara Shuzo Co., Ltd. Competent cells of *Escherichia coli* JM109 (produced by Takara Shuzo) were transformed with the ligated DNA to obtain transformant strains.

[0043] Plasmids were prepared from the transformant strains using the alkali method (Text for Bioengineering Experiments, Edited by the Society for Bioscience and Bioengineering, Japan, p.105, Baifukan, 1992) and restriction maps of the plasmids were prepared. One showing a restriction map equivalent to that shown at a lower position in Fig. 4 was designated as pYMK. pYMK had a size of about 7.0 kb, and was able to autonomously replicate in *E. coli* and coryneform bacteria and impart Km resistance to a host.

Example 4

Construction of shuttle vector pYMC containing Cm resistance gene derived from Tn9

[0044] A region containing the replication origin was amplified in the same manner as in Example 2 by using pYM2 extracted from C. *thermoaminogenes* AJ12310 (FERM BP-1542) as a template and the following primers:

S1XbaI: 5'-GCT CTA GAG CAA CCA GGG GGA GGG CGC GAG GC-3' (SEQ ID NO: 14)
S3XbaI: 5'-GCT CTA GAG CTC TCG TAG GCT GCA TCG GAG GCG GGG-3' (SEQ ID NO: 15)

[0045] The above DNA was purified by using MicroSpin TM S-400 HR columns produced by Amersham Pharmacia Biotech Co., digested with a restriction enzyme *XbaI* produced by Takara Shuzo Co., Ltd., and then ligated to a fragment obtained by treating pHSG399 (Takara Shuzo Co., Ltd.) with *XbaI* by using DNA Ligation Kit. ver. 2 produced by Takara Shuzo Co., Ltd. Competent cells of *Escherichia coli* JM109 (produced by Takara Shuzo) were transformed with the ligated DNA to obtain transformant strains.

[0046] Plasmids were prepared from the transformant strains using the alkali method (Text for Bioengineering Experiments, Edited by the Society for Bioscience and Bioengineering, Japan, p.105, Baifukan, 1992) and restriction maps of the plasmids were prepared. One showing a restriction map equivalent to that shown at a lower position in Fig. 5 was designated as pYMC. pYMC had a size of about 6.6 kb, and was able to autonomously replicate in *E. coli* and coryneform bacteria and impart Cm resistance to a host.

SEQUENCE LISTING

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<120> Plasmid Autonomously Replicable in Coryneform Bacteria

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<150> JP 11-228391
<151> 1999-08-12

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<170> PatentIn Ver. 2.0

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Tyr Ser Thr Asp Leu Phe Asp Thr His Pro Glu Leu Ala Leu Arg Ser
             20                  25                  30
cgg ggt tgg aat cac cag gac gcc gcc gag ttc ctg gcc cac ctg gat    144
Arg Gly Trp Asn His Gln Asp Ala Ala Glu Phe Leu Ala His Leu Asp
         35                  40                  45
cgc agc atg ttt cac ggg tgc ccc acc cgg gat ttc tcc gcg gcc tgg    192
Arg Ser Met Phe His Gly Cys Pro Thr Arg Asp Phe Ser Ala Ala Trp
     50                  55                  60
gtc aaa gac ccg gaa acc gga gaa acc cgc ccc aag ctg cac aga gtt    240
Val Lys Asp Pro Glu Thr Gly Glu Thr Arg Pro Lys Leu His Arg Val
 65                  70                  75                  80
ggc acc cgc tca ctt tcc cgg tgc cag tac gtt gcc ctg acc cac ccg    288
Gly Thr Arg Ser Leu Ser Arg Cys Gln Tyr Val Ala Leu Thr His Pro
                 85                  90                  95
cag cgc tcc gcg gtg ctg gtc tta gac atc gac atc ccc agc cac cag    336
Gln Arg Ser Ala Val Leu Val Leu Asp Ile Asp Ile Pro Ser His Gln
            100                 105                 110
gcc ggc ggg aac atc gag cac ctt cac ccg cag gtg tac gcc acc ttg    384
Ala Gly Gly Asn Ile Glu His Leu His Pro Gln Val Tyr Ala Thr Leu
        115                 120                 125
gag cgt tgg gca cgg gtg gag aaa gcg ccg gcc tgg atc ggg gtg aac    432
Glu Arg Trp Ala Arg Val Glu Lys Ala Pro Ala Trp Ile Gly Val Asn
```

```
    130                 135                 140
ccg ttg tcg gga aag tgc cag ctc atc tgg tgc att gac ccg gtg ttc     480
Pro Leu Ser Gly Lys Cys Gln Leu Ile Trp Cys Ile Asp Pro Val Phe
145                 150                 155                 160
gcc gcc gag ggc acc acc agc tcg aac acc cgc ctg cta gcg gcc acc     528
Ala Ala Glu Gly Thr Thr Ser Ser Asn Thr Arg Leu Leu Ala Ala Thr
                165                 170                 175
acc gag gaa atg acc cgt gtg ttc ggc gct gac cag gca ttt tcc cac     576
Thr Glu Glu Met Thr Arg Val Phe Gly Ala Asp Gln Ala Phe Ser His
            180                 185                 190
cgg ctg agc cgg tgg ccg ctg cat gtt tct gat gat ccg acc gcg tac     624
Arg Leu Ser Arg Trp Pro Leu His Val Ser Asp Asp Pro Thr Ala Tyr
        195                 200                 205
tcc tgg cac tgc cag cac aac cga gtc gat att ctt gat gag ctg atg     672
Ser Trp His Cys Gln His Asn Arg Val Asp Ile Leu Asp Glu Leu Met
    210                 215                 220
gag gta gcc cgc acg atg acc gga tca aaa aag ccc aga gag cac gct     720
Glu Val Ala Arg Thr Met Thr Gly Ser Lys Lys Pro Arg Glu His Ala
225                 230                 235                 240
cac cag gag ttt tcc agc ggt cgg gca cgg atc gaa gcc gcg cgg aaa     768
His Gln Glu Phe Ser Ser Gly Arg Ala Arg Ile Glu Ala Ala Arg Lys
                245                 250                 255
gcc acc gca gag gcc aaa gcg ctt gcc gcc ttg gac gcc acg ctg cct     816
Ala Thr Ala Glu Ala Lys Ala Leu Ala Ala Leu Asp Ala Thr Leu Pro
            260                 265                 270
acg gcg ctg gag gca tca ggc gat ctc att gac ggg gtg cgg gtg ttg     864
Thr Ala Leu Glu Ala Ser Gly Asp Leu Ile Asp Gly Val Arg Val Leu
        275                 280                 285
tgg gca gca gag ggg cgt gca gcc cgt gat gag aca gcg ttt cgc cat     912
Trp Ala Ala Glu Gly Arg Ala Ala Arg Asp Glu Thr Ala Phe Arg His
    290                 295                 300
gcg ttg acc gtg ggt tat cag ctt aaa gcc gca ggt gaa cgc ctg aaa     960
Ala Leu Thr Val Gly Tyr Gln Leu Lys Ala Ala Gly Glu Arg Leu Lys
305                 310                 315                 320
gat gcc aag atc att gat gcg tat gag cgt gcc tac aac gtc gcc cag     1008
Asp Ala Lys Ile Ile Asp Ala Tyr Glu Arg Ala Tyr Asn Val Ala Gln
                325                 330                 335
gcg gtg gga gct gat ggg cgt gaa ccg gat ctg cct gcc atg cgt gat     1056
Ala Val Gly Ala Asp Gly Arg Glu Pro Asp Leu Pro Ala Met Arg Asp
            340                 345                 350
cgt cag acg atg gcc cgc cgt gtg cgc gcc tac gtc gcc aaa ggc cag     1104
Arg Gln Thr Met Ala Arg Arg Val Arg Ala Tyr Val Ala Lys Gly Gln
        355                 360                 365
ccc acg gtc agc gcc agg agc aca cag acc cag agc agt cgg ggc cgg     1152
Pro Thr Val Ser Ala Arg Ser Thr Gln Thr Gln Ser Ser Arg Gly Arg
    370                 375                 380
aaa gcc ctg gcc acc atg ggc cgc aga ggc ggg caa aaa gcc gct gaa     1200
Lys Ala Leu Ala Thr Met Gly Arg Arg Gly Gly Gln Lys Ala Ala Glu
385                 390                 395                 400
cgc tgg aaa acc gat cct aac ggc aaa tac gcc caa gaa aac cgc caa     1248
Arg Trp Lys Thr Asp Pro Asn Gly Lys Tyr Ala Gln Glu Asn Arg Gln
                405                 410                 415
cga ctc gaa gct gca aac aag cga cgt caa gtc agc tgg aac aaa tac     1296
Arg Leu Glu Ala Ala Asn Lys Arg Arg Gln Val Ser Trp Asn Lys Tyr
            420                 425                 430
gcg agc acg aat tct ggc tac ggt ttc cga cac gta tgg gcc agc ttg     1344
```

```
Ala Ser Thr Asn Ser Gly Tyr Gly Phe Arg His Val Trp Ala Ser Leu
        435                 440                 445
gaa aaa tgc cta cgc gac gag caa atc atg gaa gaa aca ggg ctt tca     1392
Glu Lys Cys Leu Arg Asp Glu Gln Ile Met Glu Glu Thr Gly Leu Ser
    450                 455                 460
cga gct acc gtg acg cgc cat tgg gtg cac tgc gag agg ctg gcc tgc     1440
Arg Ala Thr Val Thr Arg His Trp Val His Cys Glu Arg Leu Ala Cys
465                 470                 475                 480
tgc caa atc ctt agg ggg gct cac gcc gta gac aga taa                 1479
Cys Gln Ile Leu Arg Gly Ala His Ala Val Asp Arg
                485                 490
```

<210> 2
<211> 492
<212> PRT
<213> Corynebacterium thermoaminogenes

<400> 2

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Met Thr Leu Ala Asp Ser Pro Gly Thr Tyr Thr Ala Asp Ala Trp Asn
1               5                   10                  15
Tyr Ser Thr Asp Leu Phe Asp Thr His Pro Glu Leu Ala Leu Arg Ser
            20                  25                  30
Arg Gly Trp Asn His Gln Asp Ala Ala Glu Phe Leu Ala His Leu Asp
        35                  40                  45
Arg Ser Met Phe His Gly Cys Pro Thr Arg Asp Phe Ser Ala Ala Trp
    50                  55                  60
Val Lys Asp Pro Glu Thr Gly Glu Thr Arg Pro Lys Leu His Arg Val
65                  70                  75                  80
Gly Thr Arg Ser Leu Ser Arg Cys Gln Tyr Val Ala Leu Thr His Pro
                85                  90                  95
Gln Arg Ser Ala Val Leu Val Leu Asp Ile Asp Ile Pro Ser His Gln
            100                 105                 110
Ala Gly Gly Asn Ile Glu His Leu His Pro Gln Val Tyr Ala Thr Leu
        115                 120                 125
Glu Arg Trp Ala Arg Val Glu Lys Ala Pro Ala Trp Ile Gly Val Asn
    130                 135                 140
Pro Leu Ser Gly Lys Cys Gln Leu Ile Trp Cys Ile Asp Pro Val Phe
145                 150                 155                 160
Ala Ala Glu Gly Thr Thr Ser Ser Asn Thr Arg Leu Leu Ala Ala Thr
                165                 170                 175
Thr Glu Glu Met Thr Arg Val Phe Gly Ala Asp Gln Ala Phe Ser His
            180                 185                 190
Arg Leu Ser Arg Trp Pro Leu His Val Ser Asp Asp Pro Thr Ala Tyr
        195                 200                 205
Ser Trp His Cys Gln His Asn Arg Val Asp Ile Leu Asp Glu Leu Met
    210                 215                 220
Glu Val Ala Arg Thr Met Thr Gly Ser Lys Lys Pro Arg Glu His Ala
225                 230                 235                 240
His Gln Glu Phe Ser Ser Gly Arg Ala Arg Ile Glu Ala Ala Arg Lys
                245                 250                 255
Ala Thr Ala Glu Ala Lys Ala Leu Ala Ala Leu Asp Ala Thr Leu Pro
            260                 265                 270
Thr Ala Leu Glu Ala Ser Gly Asp Leu Ile Asp Gly Val Arg Val Leu
        275                 280                 285
Trp Ala Ala Glu Gly Arg Ala Ala Arg Asp Glu Thr Ala Phe Arg His
    290                 295                 300
```

Ala Leu Thr Val Gly Tyr Gln Leu Lys Ala Ala Gly Glu Arg Leu Lys
305 310 315 320
Asp Ala Lys Ile Ile Asp Ala Tyr Glu Arg Ala Tyr Asn Val Ala Gln
325 330 335
Ala Val Gly Ala Asp Gly Arg Glu Pro Asp Leu Pro Ala Met Arg Asp
340 345 350
Arg Gln Thr Met Ala Arg Arg Val Arg Ala Tyr Val Ala Lys Gly Gln
355 360 365
Pro Thr Val Ser Ala Arg Ser Thr Gln Thr Gln Ser Ser Arg Gly Arg
370 375 380
Lys Ala Leu Ala Thr Met Gly Arg Arg Gly Gly Gln Lys Ala Ala Glu
385 390 395 400
Arg Trp Lys Thr Asp Pro Asn Gly Lys Tyr Ala Gln Glu Asn Arg Gln
405 410 415
Arg Leu Glu Ala Ala Asn Lys Arg Arg Gln Val Ser Trp Asn Lys Tyr
420 425 430
Ala Ser Thr Asn Ser Gly Tyr Gly Phe Arg His Val Trp Ala Ser Leu
435 440 445
Glu Lys Cys Leu Arg Asp Glu Gln Ile Met Glu Glu Thr Gly Leu Ser
450 455 460
Arg Ala Thr Val Thr Arg His Trp Val His Cys Glu Arg Leu Ala Cys
465 470 475 480
Cys Gln Ile Leu Arg Gly Ala His Ala Val Asp Arg
485 490

<210> 3
<211> 1479
<212> DNA
<213> Corynebacterium thermoaminogenes

<220>
<221> CDS
<222> (1)..(1476)

<400> 3
atg act cta gcg gat tcg cca gga aca tac aca gca gat gcg tgg aat 48
Met Thr Leu Ala Asp Ser Pro Gly Thr Tyr Thr Ala Asp Ala Trp Asn
1 5 10 15
tac tcc act gat ctg ttc gac acc cac cct gag ctg gct tta cgc tcc 96
Tyr Ser Thr Asp Leu Phe Asp Thr His Pro Glu Leu Ala Leu Arg Ser
20 25 30
cgg ggt tgg aat cac cag gac gcc gca gag ttc ctg gcc cac ctg gat 144
Arg Gly Trp Asn His Gln Asp Ala Ala Glu Phe Leu Ala His Leu Asp
35 40 45
cgc agc atg ttt cac ggg tgc ccc acc cgg gat ttc tcc gcg gcc tgg 192
Arg Ser Met Phe His Gly Cys Pro Thr Arg Asp Phe Ser Ala Ala Trp
50 55 60
gtc aaa gac ccg gaa acc gga gaa acc cgc ccc aag ctg cac aga gtt 240
Val Lys Asp Pro Glu Thr Gly Glu Thr Arg Pro Lys Leu His Arg Val
65 70 75 80
ggc acc cgc tca ctt tcc cgg tgc cag tac gtt gcc ctg acc cac ccg 288
Gly Thr Arg Ser Leu Ser Arg Cys Gln Tyr Val Ala Leu Thr His Pro
85 90 95
cag cgc tcc gcg gtg ctg gtc tta gac atc gac atc ccc agc cac cag 336
Gln Arg Ser Ala Val Leu Val Leu Asp Ile Asp Ile Pro Ser His Gln
100 105 110

```
gcc ggc ggg aac atc gag cac ctt cac ccg cag gtg tac gcc acc ttg    384
Ala Gly Gly Asn Ile Glu His Leu His Pro Gln Val Tyr Ala Thr Leu
        115                 120                 125
gag cgt tgg gca cgg gtg gag aaa gcg ccg gcc tgg atc ggg gtg aac    432
Glu Arg Trp Ala Arg Val Glu Lys Ala Pro Ala Trp Ile Gly Val Asn
    130                 135                 140
ccg ttg tcg gga aag tgc cag ctc atc tgg tgc att gac ccg gtg ttc    480
Pro Leu Ser Gly Lys Cys Gln Leu Ile Trp Cys Ile Asp Pro Val Phe
145                 150                 155                 160
gcc gcc gag ggc acc acc agc tcg aac acc cgc ctg cta gcg gcc acc    528
Ala Ala Glu Gly Thr Thr Ser Ser Asn Thr Arg Leu Leu Ala Ala Thr
                165                 170                 175
acc gag gaa atg acc cgt gtg ttc ggc gct gac cag gca ttt tcc cac    576
Thr Glu Glu Met Thr Arg Val Phe Gly Ala Asp Gln Ala Phe Ser His
            180                 185                 190
cgg ctg agc cgg tgg ccg ctg cat gtt ttt gat gat ccg acc gcg tac    624
Arg Leu Ser Arg Trp Pro Leu His Val Phe Asp Asp Pro Thr Ala Tyr
        195                 200                 205
tcc tgg cac tgc cag cac aac cga gtc gat att ctt gat gag ctg atg    672
Ser Trp His Cys Gln His Asn Arg Val Asp Ile Leu Asp Glu Leu Met
    210                 215                 220
gag gta gcc cgc acg atg acc gga tca aaa aag ccg aga aag cac gct    720
Glu Val Ala Arg Thr Met Thr Gly Ser Lys Lys Pro Arg Lys His Ala
225                 230                 235                 240
cac cag gag ttt tcc agc ggt cgg gca cgg atc gaa gcc gcg cgg aaa    768
His Gln Glu Phe Ser Ser Gly Arg Ala Arg Ile Glu Ala Ala Arg Lys
                245                 250                 255
gcc acc gca gag gcc aaa gcg ctt gcc gcc ttg gac gcc acg ctg cct    816
Ala Thr Ala Glu Ala Lys Ala Leu Ala Ala Leu Asp Ala Thr Leu Pro
            260                 265                 270
acg gcg ctg gag gca tca ggc gat ctc att gac ggg gtg cgg gtg ttg    864
Thr Ala Leu Glu Ala Ser Gly Asp Leu Ile Asp Gly Val Arg Val Leu
        275                 280                 285
tgg gca gca gag ggg cgt gca gcc cgt gat gag aca gcg ttt cgc cat    912
Trp Ala Ala Glu Gly Arg Ala Ala Arg Asp Glu Thr Ala Phe Arg His
    290                 295                 300
gcg ttg acc gtg ggt tat cag ctt aaa gcc gca ggt gaa cgc ctg aaa    960
Ala Leu Thr Val Gly Tyr Gln Leu Lys Ala Ala Gly Glu Arg Leu Lys
305                 310                 315                 320
gat gcc aag atc att gat gcg tat gag cgt gcc tac aac gtc gcc cag    1008
Asp Ala Lys Ile Ile Asp Ala Tyr Glu Arg Ala Tyr Asn Val Ala Gln
                325                 330                 335
gcg gtg gga gct gat ggg cgt gaa ccg gat ctg cct gcc atg cgt gat    1056
Ala Val Gly Ala Asp Gly Arg Glu Pro Asp Leu Pro Ala Met Arg Asp
            340                 345                 350
cgt cag acg atg gcc cgc cgt gtg cgc gcc tac gtc gcc aaa ggc cag    1104
Arg Gln Thr Met Ala Arg Arg Val Arg Ala Tyr Val Ala Lys Gly Gln
        355                 360                 365
ccc acg gtc agc gcc agg agc aca cag acc cag agc agt cgg ggc cgg    1152
Pro Thr Val Ser Ala Arg Ser Thr Gln Thr Gln Ser Ser Arg Gly Arg
    370                 375                 380
aaa gcc ctg gcc acc atg ggc cgc aga ggc ggg caa aaa gcc gct gaa    1200
Lys Ala Leu Ala Thr Met Gly Arg Arg Gly Gly Gln Lys Ala Ala Glu
385                 390                 395                 400
cgc tgg aaa acc gat cct aac ggc aaa tac gcc caa gaa aac cgc caa    1248
Arg Trp Lys Thr Asp Pro Asn Gly Lys Tyr Ala Gln Glu Asn Arg Gln
```

```
                    405                           410                          415
cga ctc gaa gct gca aac aag cga cgt caa gtc agc tgg aac aaa tac       1296
Arg Leu Glu Ala Ala Asn Lys Arg Arg Gln Val Ser Trp Asn Lys Tyr
                420                           425                          430
gcg agc acg aat tct ggc tac ggt ttc cga cac gta tgg gcc agc ttg       1344
Ala Ser Thr Asn Ser Gly Tyr Gly Phe Arg His Val Trp Ala Ser Leu
            435                           440                          445
gaa aaa tgc cta cgc gac gag caa atc atg gaa gaa aca ggg ctt tca       1392
Glu Lys Cys Leu Arg Asp Glu Gln Ile Met Glu Glu Thr Gly Leu Ser
        450                           455                          460
cga gct acc gtg acg cgc cat tgg gtg cac tgc gag agg ctg gcc tgc       1440
Arg Ala Thr Val Thr Arg His Trp Val His Cys Glu Arg Leu Ala Cys
465                           470                          475                          480
tgc caa atc ctt agg ggg gct cac gcc gta cac aga taa                    1479
Cys Gln Ile Leu Arg Gly Ala His Ala Val His Arg
                485                           490
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<210> 4
<211> 492
<212> PRT
<213> Corynebacterium thermoaminogenes

<400> 4

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Met Thr Leu Ala Asp Ser Pro Gly Thr Tyr Thr Ala Asp Ala Trp Asn
 1               5                   10                  15
Tyr Ser Thr Asp Leu Phe Asp Thr His Pro Glu Leu Ala Leu Arg Ser
            20                  25                  30
Arg Gly Trp Asn His Gln Asp Ala Ala Glu Phe Leu Ala His Leu Asp
        35                  40                  45
Arg Ser Met Phe His Gly Cys Pro Thr Arg Asp Phe Ser Ala Ala Trp
    50                  55                  60
Val Lys Asp Pro Glu Thr Gly Glu Thr Arg Pro Lys Leu His Arg Val
65                  70                  75                  80
Gly Thr Arg Ser Leu Ser Arg Cys Gln Tyr Val Ala Leu Thr His Pro
                85                  90                  95
Gln Arg Ser Ala Val Leu Val Leu Asp Ile Asp Ile Pro Ser His Gln
            100                 105                 110
Ala Gly Gly Asn Ile Glu His Leu His Pro Gln Val Tyr Ala Thr Leu
        115                 120                 125
Glu Arg Trp Ala Arg Val Glu Lys Ala Pro Ala Trp Ile Gly Val Asn
    130                 135                 140
Pro Leu Ser Gly Lys Cys Gln Leu Ile Trp Cys Ile Asp Pro Val Phe
145                 150                 155                 160
Ala Ala Glu Gly Thr Thr Ser Ser Asn Thr Arg Leu Leu Ala Ala Thr
                165                 170                 175
Thr Glu Glu Met Thr Arg Val Phe Gly Ala Asp Gln Ala Phe Ser His
            180                 185                 190
Arg Leu Ser Arg Trp Pro Leu His Val Phe Asp Asp Pro Thr Ala Tyr
        195                 200                 205
Ser Trp His Cys Gln His Asn Arg Val Asp Ile Leu Asp Glu Leu Met
    210                 215                 220
Glu Val Ala Arg Thr Met Thr Gly Ser Lys Lys Pro Arg Lys His Ala
225                 230                 235                 240
His Gln Glu Phe Ser Ser Gly Arg Ala Arg Ile Glu Ala Ala Arg Lys
                245                 250                 255
Ala Thr Ala Glu Ala Lys Ala Leu Ala Ala Leu Asp Ala Thr Leu Pro
```

260 265 270
Thr Ala Leu Glu Ala Ser Gly Asp Leu Ile Asp Gly Val Arg Val Leu
275 280 285
Trp Ala Ala Glu Gly Arg Ala Ala Arg Asp Glu Thr Ala Phe Arg His
290 295 300
Ala Leu Thr Val Gly Tyr Gln Leu Lys Ala Ala Gly Glu Arg Leu Lys
305 310 315 320
Asp Ala Lys Ile Ile Asp Ala Tyr Glu Arg Ala Tyr Asn Val Ala Gln
325 330 335
Ala Val Gly Ala Asp Gly Arg Glu Pro Asp Leu Pro Ala Met Arg Asp
340 345 350
Arg Gln Thr Met Ala Arg Arg Val Arg Ala Tyr Val Ala Lys Gly Gln
355 360 365
Pro Thr Val Ser Ala Arg Ser Thr Gln Thr Gln Ser Ser Arg Gly Arg
370 375 380
Lys Ala Leu Ala Thr Met Gly Arg Arg Gly Gly Gln Lys Ala Ala Glu
385 390 395 400
Arg Trp Lys Thr Asp Pro Asn Gly Lys Tyr Ala Gln Glu Asn Arg Gln
405 410 415
Arg Leu Glu Ala Ala Asn Lys Arg Arg Gln Val Ser Trp Asn Lys Tyr
420 425 430
Ala Ser Thr Asn Ser Gly Tyr Gly Phe Arg His Val Trp Ala Ser Leu
435 440 445
Glu Lys Cys Leu Arg Asp Glu Gln Ile Met Glu Glu Thr Gly Leu Ser
450 455 460
Arg Ala Thr Val Thr Arg His Trp Val His Cys Glu Arg Leu Ala Cys
465 470 475 480
Cys Gln Ile Leu Arg Gly Ala His Ala Val His Arg
485 490

<210> 5
<211> 1479
<212> DNA
<213> Corynebacterium thermoaminogenes

<220>
<221> CDS
<222> (1)..(1476)

<400> 5
atg act cta gcg gat tcg cca gga aca tac aca gca gat gcg tgg aat 48
Met Thr Leu Ala Asp Ser Pro Gly Thr Tyr Thr Ala Asp Ala Trp Asn
1 5 10 15
tac tcc act gat ctg ttc gac acc cac cct gag ctg gct tta cgc tcc 96
Tyr Ser Thr Asp Leu Phe Asp Thr His Pro Glu Leu Ala Leu Arg Ser
20 25 30
cgg ggt tgg aat cac cag gac gcc gcc gag ttc ctg gcc cac ctg gat 144
Arg Gly Trp Asn His Gln Asp Ala Ala Glu Phe Leu Ala His Leu Asp
35 40 45
cgc agc atg ttt cac ggg tgc ccc acc cgg gat ttc tcc gcg gcc tgg 192
Arg Ser Met Phe His Gly Cys Pro Thr Arg Asp Phe Ser Ala Ala Trp
50 55 60
gtc aaa gac ccg gaa acc gga gaa acc cgc ccc aag ctg cac aga gtt 240
Val Lys Asp Pro Glu Thr Gly Glu Thr Arg Pro Lys Leu His Arg Val
65 70 75 80
ggc acc cgc tca ctt tcc cgg tgc cag tac gtt gcc ctg acc cac ccg 288

```
Gly Thr Arg Ser Leu Ser Arg Cys Gln Tyr Val Ala Leu Thr His Pro
                85                  90                  95
cag cgc tcc gcg gtg ctg gtc tta gac atc gac atc ccc agc cac cag    336
Gln Arg Ser Ala Val Leu Val Leu Asp Ile Asp Ile Pro Ser His Gln
            100                 105                 110
gcc ggc ggg aac atc gag cac ctt cac ccg cag gta tac gcc acc ttg    384
Ala Gly Gly Asn Ile Glu His Leu His Pro Gln Val Tyr Ala Thr Leu
        115                 120                 125
gag cgt tgg gca cgg gtg gag aaa gcg ccg gcc tgg atc ggg gtg aac    432
Glu Arg Trp Ala Arg Val Glu Lys Ala Pro Ala Trp Ile Gly Val Asn
    130                 135                 140
ccg ttg tcg gga aag tgc cag ctc atc tgg tgc att gac ccg gtg ttc    480
Pro Leu Ser Gly Lys Cys Gln Leu Ile Trp Cys Ile Asp Pro Val Phe
145                 150                 155                 160
gcc gcc gag ggc acc acc agc tcg aac acc cgc ctg cta gcg gcc acc    528
Ala Ala Glu Gly Thr Thr Ser Ser Asn Thr Arg Leu Leu Ala Ala Thr
                165                 170                 175
acc gag gaa atg acc cgt gtg ttc ggc gct gac cag gca ttt tcc cac    576
Thr Glu Glu Met Thr Arg Val Phe Gly Ala Asp Gln Ala Phe Ser His
            180                 185                 190
cgg ctg agc cgg tgg ccg ctg cat gtt tct gat gat ccg acc gcg tac    624
Arg Leu Ser Arg Trp Pro Leu His Val Ser Asp Asp Pro Thr Ala Tyr
        195                 200                 205
tcc tgg cac tgc cag cac aac cga gtc gat acg ctt gat gag ctg atg    672
Ser Trp His Cys Gln His Asn Arg Val Asp Thr Leu Asp Glu Leu Met
    210                 215                 220
gag gta gcc cgc acg atg acc gga tca aaa aag ccg aga aag cac gct    720
Glu Val Ala Arg Thr Met Thr Gly Ser Lys Lys Pro Arg Lys His Ala
225                 230                 235                 240
cac cag gag ttt tcc agc ggt cgg gca cgg atc gaa gcc gcg cgg aaa    768
His Gln Glu Phe Ser Ser Gly Arg Ala Arg Ile Glu Ala Ala Arg Lys
                245                 250                 255
gcc acc gca gag gcc aaa gcg ctt gcc gcc ttg gac gcc acg ctg cct    816
Ala Thr Ala Glu Ala Lys Ala Leu Ala Ala Leu Asp Ala Thr Leu Pro
            260                 265                 270
acg gcg ctg gag gca tca ggc gat ctc att gac ggg gtg cgg gtg ttg    864
Thr Ala Leu Glu Ala Ser Gly Asp Leu Ile Asp Gly Val Arg Val Leu
        275                 280                 285
tgg gca gca gag ggg cgt gca gcc cgt gat gag aca gcg ttt cgc cat    912
Trp Ala Ala Glu Gly Arg Ala Ala Arg Asp Glu Thr Ala Phe Arg His
    290                 295                 300
gcg ttg acc gtg ggt tat cag ctt aaa gcc gca ggt gaa cgc ctg aaa    960
Ala Leu Thr Val Gly Tyr Gln Leu Lys Ala Ala Gly Glu Arg Leu Lys
305                 310                 315                 320
gat gcc aag atc att gat gcg tat gag cgt gcc tac aac gtc gcc cag   1008
Asp Ala Lys Ile Ile Asp Ala Tyr Glu Arg Ala Tyr Asn Val Ala Gln
                325                 330                 335
gcg gtg gga gct gat ggg cgt gaa ccg gat ctg cct gcc atg cgt gat   1056
Ala Val Gly Ala Asp Gly Arg Glu Pro Asp Leu Pro Ala Met Arg Asp
            340                 345                 350
cgt cag acg atg gcc cgc cgt gtg cgc gcc tac gtc gcc aaa ggc cag   1104
Arg Gln Thr Met Ala Arg Arg Val Arg Ala Tyr Val Ala Lys Gly Gln
        355                 360                 365
ccc acg gtc agc gcc agg agc aca cag acc cag agc agt cgg ggc cgg   1152
Pro Thr Val Ser Ala Arg Ser Thr Gln Thr Gln Ser Ser Arg Gly Arg
    370                 375                 380
```

```
aaa gcc ctg gcc acc atg ggc cgc aga ggc ggg caa aaa gcc gct gaa    1200
Lys Ala Leu Ala Thr Met Gly Arg Arg Gly Gly Gln Lys Ala Ala Glu
385                 390                 395                 400
cgc tgg aaa acc gat cct aac ggc aaa tac gcc caa gaa aac cgc caa    1248
Arg Trp Lys Thr Asp Pro Asn Gly Lys Tyr Ala Gln Glu Asn Arg Gln
                405                 410                 415
cga ctc gaa gct gca aac aag cga cgt caa gtc agc tgg aac aaa tac    1296
Arg Leu Glu Ala Ala Asn Lys Arg Arg Gln Val Ser Trp Asn Lys Tyr
            420                 425                 430
gcg agc acg aat tct ggc tac ggt ttc cga cac gta tgg gcc agc ttg    1344
Ala Ser Thr Asn Ser Gly Tyr Gly Phe Arg His Val Trp Ala Ser Leu
        435                 440                 445
gaa aaa tgc cta cgc gac gag caa atc atg gaa gaa aca ggg ctt tca    1392
Glu Lys Cys Leu Arg Asp Glu Gln Ile Met Glu Glu Thr Gly Leu Ser
    450                 455                 460
cga gct acc gtg acg cgc cat tgg gtg cac tgc gag agg ctg gcc tgc    1440
Arg Ala Thr Val Thr Arg His Trp Val His Cys Glu Arg Leu Ala Cys
465                 470                 475                 480
tgc caa atc ctt agg ggg gct cac gcc gta cac aga taa                1479
Cys Gln Ile Leu Arg Gly Ala His Ala Val His Arg
                485                 490
```

<210> 6
<211> 492
<212> PRT
<213> Corynebacterium thermoaminogenes

<400> 6

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Met Thr Leu Ala Asp Ser Pro Gly Thr Tyr Thr Ala Asp Ala Trp Asn
 1               5                  10                  15
Tyr Ser Thr Asp Leu Phe Asp Thr His Pro Glu Leu Ala Leu Arg Ser
            20                  25                  30
Arg Gly Trp Asn His Gln Asp Ala Ala Glu Phe Leu Ala His Leu Asp
        35                  40                  45
Arg Ser Met Phe His Gly Cys Pro Thr Arg Asp Phe Ser Ala Ala Trp
    50                  55                  60
Val Lys Asp Pro Glu Thr Gly Glu Thr Arg Pro Lys Leu His Arg Val
65                  70                  75                  80
Gly Thr Arg Ser Leu Ser Arg Cys Gln Tyr Val Ala Leu Thr His Pro
                85                  90                  95
Gln Arg Ser Ala Val Leu Val Leu Asp Ile Asp Ile Pro Ser His Gln
            100                 105                 110
Ala Gly Gly Asn Ile Glu His Leu His Pro Gln Val Tyr Ala Thr Leu
        115                 120                 125
Glu Arg Trp Ala Arg Val Glu Lys Ala Pro Ala Trp Ile Gly Val Asn
    130                 135                 140
Pro Leu Ser Gly Lys Cys Gln Leu Ile Trp Cys Ile Asp Pro Val Phe
145                 150                 155                 160
Ala Ala Glu Gly Thr Thr Ser Ser Asn Thr Arg Leu Leu Ala Ala Thr
                165                 170                 175
Thr Glu Glu Met Thr Arg Val Phe Gly Ala Asp Gln Ala Phe Ser His
            180                 185                 190
Arg Leu Ser Arg Trp Pro Leu His Val Ser Asp Asp Pro Thr Ala Tyr
        195                 200                 205
Ser Trp His Cys Gln His Asn Arg Val Asp Thr Leu Asp Glu Leu Met
    210                 215                 220
```

Glu Val Ala Arg Thr Met Thr Gly Ser Lys Lys Pro Arg Lys His Ala
225 230 235 240
His Gln Glu Phe Ser Ser Gly Arg Ala Arg Ile Glu Ala Ala Arg Lys
245 250 255
Ala Thr Ala Glu Ala Lys Ala Leu Ala Ala Leu Asp Ala Thr Leu Pro
260 265 270
Thr Ala Leu Glu Ala Ser Gly Asp Leu Ile Asp Gly Val Arg Val Leu
275 280 285
Trp Ala Ala Glu Gly Arg Ala Ala Arg Asp Glu Thr Ala Phe Arg His
290 295 300
Ala Leu Thr Val Gly Tyr Gln Leu Lys Ala Ala Gly Glu Arg Leu Lys
305 310 315 320
Asp Ala Lys Ile Ile Asp Ala Tyr Glu Arg Ala Tyr Asn Val Ala Gln
325 330 335
Ala Val Gly Ala Asp Gly Arg Glu Pro Asp Leu Pro Ala Met Arg Asp
340 345 350
Arg Gln Thr Met Ala Arg Arg Val Arg Ala Tyr Val Ala Lys Gly Gln
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Pro Thr Val Ser Ala Arg Ser Thr Gln Thr Gln Ser Ser Arg Gly Arg
370 375 380
Lys Ala Leu Ala Thr Met Gly Arg Arg Gly Gly Gln Lys Ala Ala Glu
385 390 395 400
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Arg Leu Glu Ala Ala Asn Lys Arg Arg Gln Val Ser Trp Asn Lys Tyr
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Ala Ser Thr Asn Ser Gly Tyr Gly Phe Arg His Val Trp Ala Ser Leu
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Glu Lys Cys Leu Arg Asp Glu Gln Ile Met Glu Glu Thr Gly Leu Ser
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Cys Gln Ile Leu Arg Gly Ala His Ala Val His Arg
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tac tcc aca gat ctg ttc gac acc cac cct gag ctg gct tta cgc tcc 96
Tyr Ser Thr Asp Leu Phe Asp Thr His Pro Glu Leu Ala Leu Arg Ser
20 25 30
cgg ggt tgg aat cac cag gac gcc gcc gag ttc ctg gcc cac ctg gat 144
Arg Gly Trp Asn His Gln Asp Ala Ala Glu Phe Leu Ala His Leu Asp
35 40 45
cgc agc atg ttt cac ggg tgc ccc acc cgg gat ttc tcc gcg gcc tgg 192
Arg Ser Met Phe His Gly Cys Pro Thr Arg Asp Phe Ser Ala Ala Trp

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gtc aaa gac ccg gag acc gga gaa acc cgc cct aag ctg cac aga gtc    240
Val Lys Asp Pro Glu Thr Gly Glu Thr Arg Pro Lys Leu His Arg Val
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ggc acc cgg tcg ctt tcc cga tgc cag tac gtc gcg ctg acc cac ccg    288
Gly Thr Arg Ser Leu Ser Arg Cys Gln Tyr Val Ala Leu Thr His Pro
                 85                  90                  95
cag cgc tcc gcg gtg ctg gtc tta gac atc gac atc ccc agc cac cag    336
Gln Arg Ser Ala Val Leu Val Leu Asp Ile Asp Ile Pro Ser His Gln
            100                 105                 110
gcc ggc ggg aac atc gag cac ctt cac ccg cag gtc tac gcc acc ttg    384
Ala Gly Gly Asn Ile Glu His Leu His Pro Gln Val Tyr Ala Thr Leu
        115                 120                 125
gag cgc tgg gca cgg gtg gag aaa gcg ccg gcc tgg atc ggg gtg aac    432
Glu Arg Trp Ala Arg Val Glu Lys Ala Pro Ala Trp Ile Gly Val Asn
    130                 135                 140
ccg ttg tca gga aag tgc cag ctc atc tgg tgc att gac ccg gtg ttc    480
Pro Leu Ser Gly Lys Cys Gln Leu Ile Trp Cys Ile Asp Pro Val Phe
145                 150                 155                 160
gcc gcc gag ggc acc acc agc ccg aac acc cgc ctg cta gcg gcc acc    528
Ala Ala Glu Gly Thr Thr Ser Pro Asn Thr Arg Leu Leu Ala Ala Thr
                165                 170                 175
acc gag gaa atg acc cgt atg ttc ggc gct gac cag gca ttt tcc cac    576
Thr Glu Glu Met Thr Arg Met Phe Gly Ala Asp Gln Ala Phe Ser His
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cgg ctg agc cgg tgg ccg ctg cat gta tct gat gat ccg acc gcg tac    624
Arg Leu Ser Arg Trp Pro Leu His Val Ser Asp Asp Pro Thr Ala Tyr
        195                 200                 205
tcc tgg cac tgc cag cac aac cga gtc gat acg ctt gct gag ctg atg    672
Ser Trp His Cys Gln His Asn Arg Val Asp Thr Leu Ala Glu Leu Met
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Glu Val Ala Arg Thr Met Thr Gly Ser Lys Lys Pro Asp Ser Thr Ala
225                 230                 235                 240
cac cag gag ttt tcc agc ggt cgg gca cgg atc gaa gcc gcg agg aaa    768
His Gln Glu Phe Ser Ser Gly Arg Ala Arg Ile Glu Ala Ala Arg Lys
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gcc acc gca gaa gcc aaa gcg ctt gct gcc tta gac gcc acg ctg cct    816
Ala Thr Ala Glu Ala Lys Ala Leu Ala Ala Leu Asp Ala Thr Leu Pro
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Thr Ala Leu Glu Ala Ser Gly Asp Leu Ile Asp Gly Val Arg Val Leu
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tgg gca gca gag ggg cgt gca gcc cgt gat gag acg gcg ttt cgc cat    912
Trp Ala Ala Glu Gly Arg Ala Ala Arg Asp Glu Thr Ala Phe Arg His
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Ala Leu Thr Val Gly Tyr Gln Leu Lys Ala Ala Gly Glu Arg Leu Lys
305                 310                 315                 320
gac acc aag atc att gat gcg tat gag cgt gcc tac aac gtc gcc cag   1008
Asp Thr Lys Ile Ile Asp Ala Tyr Glu Arg Ala Tyr Asn Val Ala Gln
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gcg gtg ggg gct gat ggg cgt gag ccg gat ctg cct gcc atg cgt gat   1056
Ala Val Gly Ala Asp Gly Arg Glu Pro Asp Leu Pro Ala Met Arg Asp
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Arg Gln Thr Leu Ala Arg Arg Val Arg Ala Tyr Val Ala Lys Gly Gln
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Pro Thr Val Ser Ala Arg Ser Thr Gln Thr Gln Ser Ser Arg Gly Arg
370 375 380
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Lys Ala Leu Ala Thr Met Gly Arg Arg Gly Ala Ala Thr Ser Asn Ala
385 390 395 400
cgc agg tgg gca gac cca gaa agc gat tac gcc cgc caa act cgg gag 1248
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405 410 415
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Arg Leu Ala Arg Ala Met Ser Phe Val His Ser Ala Gln Thr Arg Thr
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Arg Ala Gly Ser Trp Pro Thr Phe Pro Ser Ala Ser Ala Thr Val Thr
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35 40 45
Arg Ser Met Phe His Gly Cys Pro Thr Arg Asp Phe Ser Ala Ala Trp
50 55 60
Val Lys Asp Pro Glu Thr Gly Glu Thr Arg Pro Lys Leu His Arg Val
65 70 75 80
Gly Thr Arg Ser Leu Ser Arg Cys Gln Tyr Val Ala Leu Thr His Pro
85 90 95
Gln Arg Ser Ala Val Leu Val Leu Asp Ile Asp Ile Pro Ser His Gln
100 105 110
Ala Gly Gly Asn Ile Glu His Leu His Pro Gln Val Tyr Ala Thr Leu
115 120 125
Glu Arg Trp Ala Arg Val Glu Lys Ala Pro Ala Trp Ile Gly Val Asn
130 135 140
Pro Leu Ser Gly Lys Cys Gln Leu Ile Trp Cys Ile Asp Pro Val Phe
145 150 155 160
Ala Ala Glu Gly Thr Thr Ser Pro Asn Thr Arg Leu Leu Ala Ala Thr
165 170 175
Thr Glu Glu Met Thr Arg Met Phe Gly Ala Asp Gln Ala Phe Ser His
180 185 190
Arg Leu Ser Arg Trp Pro Leu His Val Ser Asp Asp Pro Thr Ala Tyr
195 200 205
Ser Trp His Cys Gln His Asn Arg Val Asp Thr Leu Ala Glu Leu Met
210 215 220
Glu Val Ala Arg Thr Met Thr Gly Ser Lys Lys Pro Asp Ser Thr Ala

225 230 235 240
His Gln Glu Phe Ser Ser Gly Arg Ala Arg Ile Glu Ala Ala Arg Lys
245 250 255
Ala Thr Ala Glu Ala Lys Ala Leu Ala Ala Leu Asp Ala Thr Leu Pro
260 265 270
Thr Ala Leu Glu Ala Ser Gly Asp Leu Ile Asp Gly Val Arg Val Leu
275 280 285
Trp Ala Ala Glu Gly Arg Ala Ala Arg Asp Glu Thr Ala Phe Arg His
290 295 300
Ala Leu Thr Val Gly Tyr Gln Leu Lys Ala Ala Gly Glu Arg Leu Lys
305 310 315 320
Asp Thr Lys Ile Ile Asp Ala Tyr Glu Arg Ala Tyr Asn Val Ala Gln
325 330 335
Ala Val Gly Ala Asp Gly Arg Glu Pro Asp Leu Pro Ala Met Arg Asp
340 345 350
Arg Gln Thr Leu Ala Arg Arg Val Arg Ala Tyr Val Ala Lys Gly Gln
355 360 365
Pro Thr Val Ser Ala Arg Ser Thr Gln Thr Gln Ser Ser Arg Gly Arg
370 375 380
Lys Ala Leu Ala Thr Met Gly Arg Arg Gly Ala Ala Thr Ser Asn Ala
385 390 395 400
Arg Arg Trp Ala Asp Pro Glu Ser Asp Tyr Ala Arg Gln Thr Arg Glu
405 410 415
Arg Leu Ala Arg Ala Met Ser Phe Val His Ser Ala Gln Thr Arg Thr
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Arg Ala Gly Ser Trp Pro Thr Phe Pro Ser Ala Ser Ala Thr Val Thr
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Thr Pro Arg Ala Lys Lys Ser Gln Arg Ser
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tac tcc act gat ctg ttc gac acc cac cct gag ctg gct tta cgc tcc 96
Tyr Ser Thr Asp Leu Phe Asp Thr His Pro Glu Leu Ala Leu Arg Ser
20 25 30
cgg ggt tgg aat cac cag gac gcc gca gag ttc ctg gcc cac ctg gat 144
Arg Gly Trp Asn His Gln Asp Ala Ala Glu Phe Leu Ala His Leu Asp
35 40 45
cgc agc atg ttt cac ggg tgc ccc acc cgg gat ttc tcc gcg gcc tgg 192
Arg Ser Met Phe His Gly Cys Pro Thr Arg Asp Phe Ser Ala Ala Trp
50 55 60
gtc aaa gac ccg gaa acc gga gaa acc cgc ccc aag ctg cac aga gtt 240
Val Lys Asp Pro Glu Thr Gly Glu Thr Arg Pro Lys Leu His Arg Val
65 70 75 80
ggc acc cgc tca ctt tcc cgg tgc cag tac gtt gcc ctg acc cac ccg 288

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Gly Thr Arg Ser Leu Ser Arg Cys Gln Tyr Val Ala Leu Thr His Pro
                85                  90                  95
cag cgc tcc gcg gtg ctg gtc tta gac atc gac atc ccc agc cac cag      336
Gln Arg Ser Ala Val Leu Val Leu Asp Ile Asp Ile Pro Ser His Gln
            100                 105                 110
gcc ggc ggg aac atc gag cac ctt cac ccg cag gtg tac gcc acc ttg      384
Ala Gly Gly Asn Ile Glu His Leu His Pro Gln Val Tyr Ala Thr Leu
        115                 120                 125
gag cgt tgg gca cgg gtg gag aaa gcg ccg gcc tgg atc ggg gtg aac      432
Glu Arg Trp Ala Arg Val Glu Lys Ala Pro Ala Trp Ile Gly Val Asn
    130                 135                 140
ccg ttg tcg gga aag tgc cag ctc atc tgg tgc att gac ccg gtg ttc      480
Pro Leu Ser Gly Lys Cys Gln Leu Ile Trp Cys Ile Asp Pro Val Phe
145                 150                 155                 160
gcc gcc gag ggc acc acc agc tcg aac acc cgc ctg cta gcg gcc acc      528
Ala Ala Glu Gly Thr Thr Ser Ser Asn Thr Arg Leu Leu Ala Ala Thr
                165                 170                 175
acc gag gaa atg acc cgt gtg ttc ggc gct gac cag gca ttt tcc cac      576
Thr Glu Glu Met Thr Arg Val Phe Gly Ala Asp Gln Ala Phe Ser His
            180                 185                 190
cgg ctg agc cgg tgg ccg ctg cat gtt ttt gat gat ccg acc gcg tac      624
Arg Leu Ser Arg Trp Pro Leu His Val Phe Asp Asp Pro Thr Ala Tyr
        195                 200                 205
tcc tgg cac tgc cag cac aac cga gtc gat att ctt gat gag ctg atg      672
Ser Trp His Cys Gln His Asn Arg Val Asp Ile Leu Asp Glu Leu Met
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gag gta gcc cgc acg atg acc gga tca aaa aag ccg aga aag cac gct      720
Glu Val Ala Arg Thr Met Thr Gly Ser Lys Lys Pro Arg Lys His Ala
225                 230                 235                 240
cac cag gag ttt tcc agc ggt cgg gca cgg atc gaa gcc gcg cgg aaa      768
His Gln Glu Phe Ser Ser Gly Arg Ala Arg Ile Glu Ala Ala Arg Lys
                245                 250                 255
gcc acc gca gag gcc aaa gcg ctt gcc gcc ttg gac gcc acg ctg cct      816
Ala Thr Ala Glu Ala Lys Ala Leu Ala Ala Leu Asp Ala Thr Leu Pro
            260                 265                 270
acg gcg ctg gag gca tca ggc gat ctc att gac ggg gtg cgg gtg ttg      864
Thr Ala Leu Glu Ala Ser Gly Asp Leu Ile Asp Gly Val Arg Val Leu
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tgg gca gca gag ggg cgt gca gcc cgt gat gag aca gcg ttt cgc cat      912
Trp Ala Ala Glu Gly Arg Ala Ala Arg Asp Glu Thr Ala Phe Arg His
    290                 295                 300
gcg ttg acc gtg ggt tat cag ctt aaa gcc gca ggt gaa cgc ctg aaa      960
Ala Leu Thr Val Gly Tyr Gln Leu Lys Ala Ala Gly Glu Arg Leu Lys
305                 310                 315                 320
gat gcc aag atc att gat gcg tat gag cgt gcc tac aac gtc gcc cag     1008
Asp Ala Lys Ile Ile Asp Ala Tyr Glu Arg Ala Tyr Asn Val Ala Gln
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gcg gtg gga gct gat ggg cgt gaa ccg gat ctg cct gcc atg cgt gat     1056
Ala Val Gly Ala Asp Gly Arg Glu Pro Asp Leu Pro Ala Met Arg Asp
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cgt cag acg atg gcc cgc cgt gtg cgc gcc tac gtc gcc aaa ggc cag     1104
Arg Gln Thr Met Ala Arg Arg Val Arg Ala Tyr Val Ala Lys Gly Gln
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ccc acg gtc agc gcc agg agc aca cag acc cag agc agt cgg ggc cgg     1152
Pro Thr Val Ser Ala Arg Ser Thr Gln Thr Gln Ser Ser Arg Gly Arg
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Lys Ala Leu Ala Thr Met Gly Arg Arg Gly Gly Gln Lys Ala Ala Glu
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cgc tgg aaa acc gat cct aac ggc aaa tac gcc caa gaa aac cgc caa    1248
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            405                 410                 415
cga ctc gaa gct gca aac aag cga cgt caa gtc agc tgg aac aaa tac    1296
Arg Leu Glu Ala Ala Asn Lys Arg Arg Gln Val Ser Trp Asn Lys Tyr
        420                 425                 430
gcg agc acg aat tct ggc tac ggt ttc cga cac gta tgg gcc agc ttg    1344
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gaa aaa tgc cta cgc gac gag caa atc atg gaa gaa aca ggg ctt tca    1392
Glu Lys Cys Leu Arg Asp Glu Gln Ile Met Glu Glu Thr Gly Leu Ser
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cga gct acc gtg acg cgc cat tgg gtg cac tgc gag agg ctg gcc tgc    1440
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Cys Gln Ile Leu Arg Gly Ala His Ala Val His Arg
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<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer for amplifying replication origin of pYM2

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<210> 11
<211> 26
<212> DNA
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<220>
<223> Description of Artificial Sequence: primer for amplifying replication origin of pYM2

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<210> 12
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<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer for amplifying replication origin of pYM2

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<212> DNA
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: primer for amplifying replication origin of pYM2

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<210> 15
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<212> DNA
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<220>
<223> Description of Artificial Sequence: primer for amplifying replication origin of pYM2

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<210> 16
<211> 32
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:primer for amplifying kanamycin resistant gene of Streptococcus faecalis

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<210> 17
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<212> DNA
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<220>
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<220>
<223> Description of Artificial Sequence: primer for amplifying Escherichia coli cloning vector pHSG399

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<210> 19
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer for amplifying Escherichia coli cloning vector pHSG399

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Claims

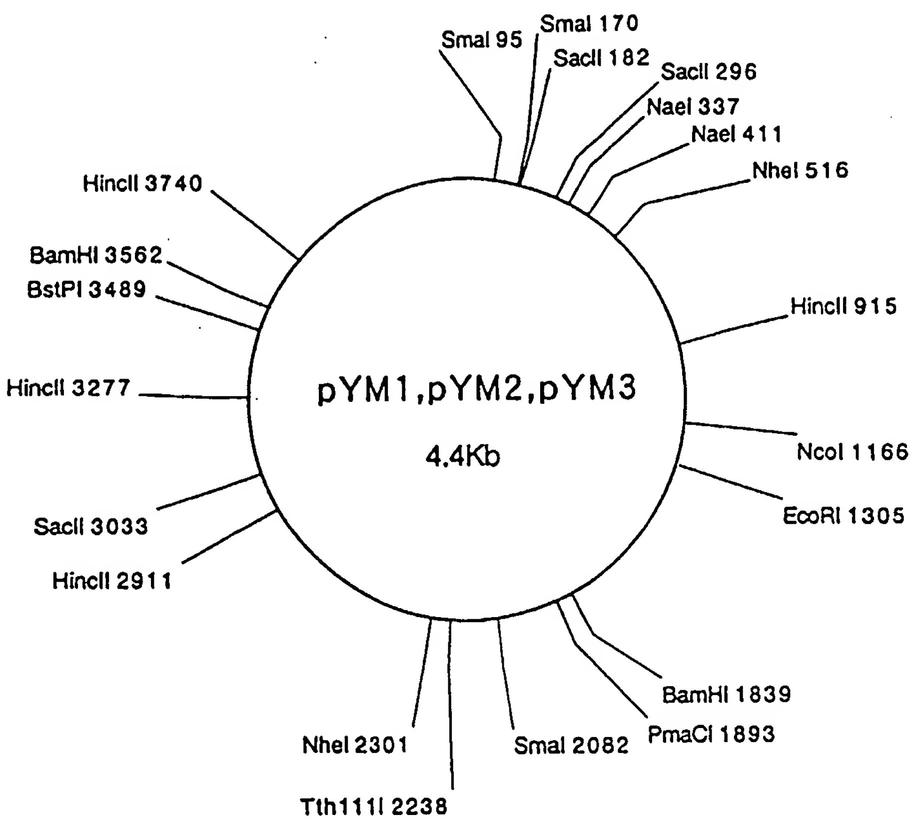
1. A plasmid isolable from *Corynebacterium thermoaminogenes*, which comprises a gene coding for a Rep protein having the amino acid sequence shown in SEQ ID NO: 2 or an amino acid sequence having homology of 90% or more to the amino acid sequence shown in SEQ ID NO: 2, and has a size of about 4.4 kb or about 6 kb, or a derivative thereof.

2. The plasmid or the derivative thereof according to claim 1, which is isolable from *Corynebacterium thermoaminogenes* AJ12340 (FERM BP-1539), AJ12308 (FERM BP-1540) or AJ12310 (FERM BP-1542), has a size of about 4.4 kb and is represented by the restriction map shown in Fig. 1.

3. The plasmid or the derivative thereof according to claim 1, which is isolable from *Corynebacterium thermoaminogenes* AJ12309 (FERM BP-1541), has a size of about 6 kb and is represented by the restriction map shown in Fig. 2.

4. The plasmid or the derivative thereof according to claim 1, which comprises a gene coding for a Rep protein having the amino acid sequence shown in SEQ ID NO: 2, 4 or 6.

5. The plasmid or the derivative thereof according to claim 1, which comprises a gene coding for a Rep protein having the amino acid sequence shown in SEQ ID NO: 8.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Sacll 182
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PmaCl 1893
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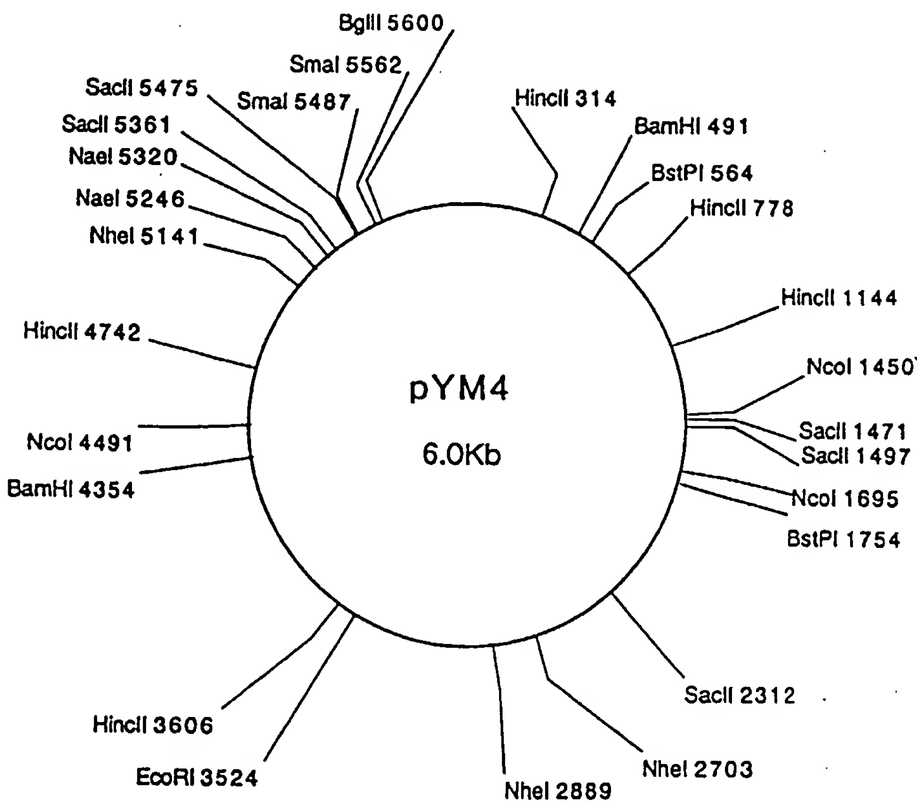
4.4Kb

Fig. 1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NaeI 5320
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BamHI 491
BstPI 564
HincII 778
HincII 1144
HincII 4742
pYM4
6.0Kb
NcoI 1450
NcoI 4491
SacII 1471
SacII 1497
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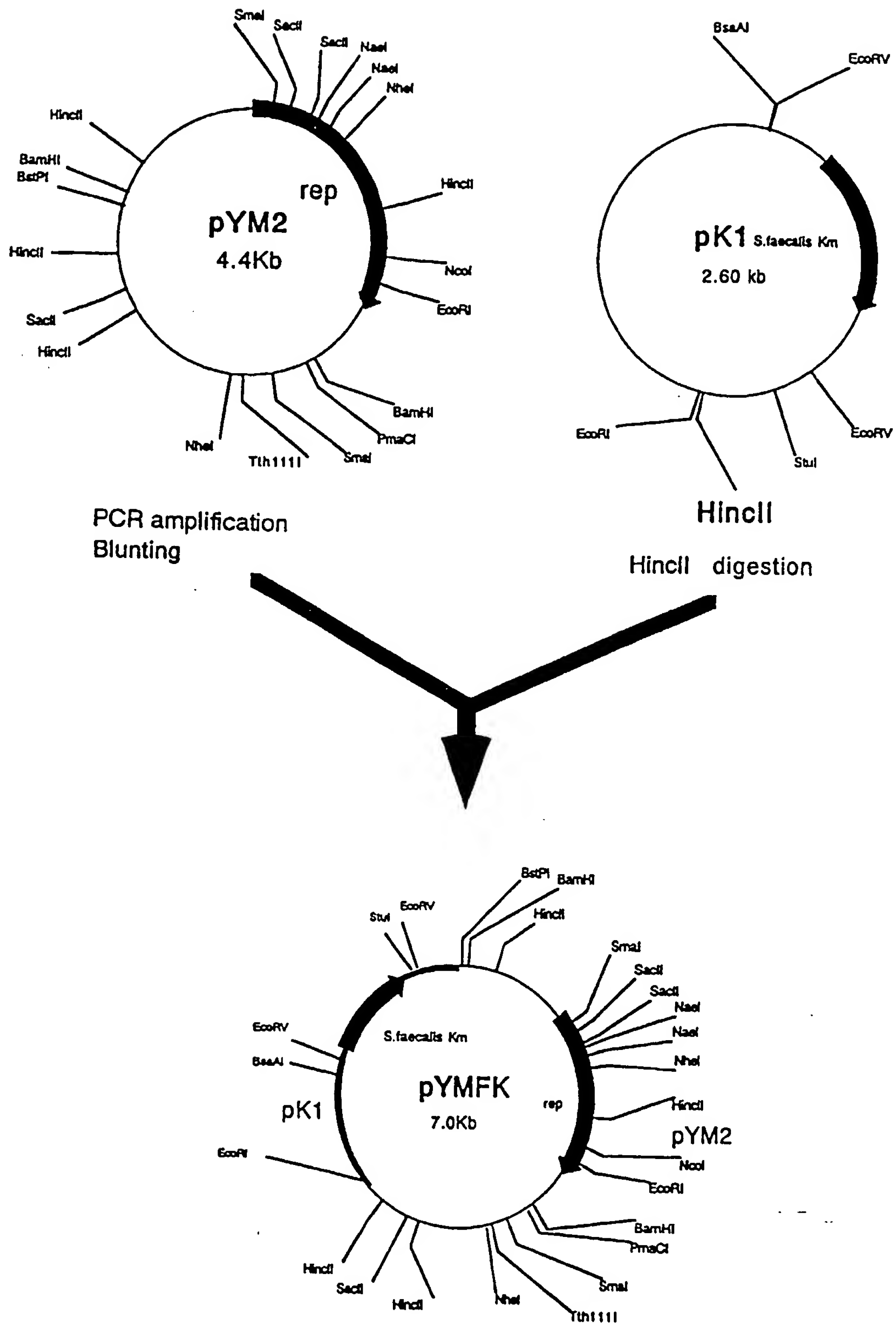
HincII 3606
EcoRI 3524
NheI 2703
NheI 2889

Fig. 2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4.4Kb
HincII
NcoI
EcoRI
BamHI
PmaCI
SmaI
NheI
Tth111I
BsaAI
EcoRV
pK1 S.faecalis Km
2.60 kb
EcoRI
StuI
EcoRV
HincII
PCR amplification
Blunting
HincII digestion
BstPI
BamHI
HincII
StuI
EcoRV
SmaI
SacII
SacII
NaeI
NaeI
NheI
EcoRV
BsaAI
S.faecalis Km
pYMFK
7.0Kb
rep
pK1
HincII
pYM2
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HincII
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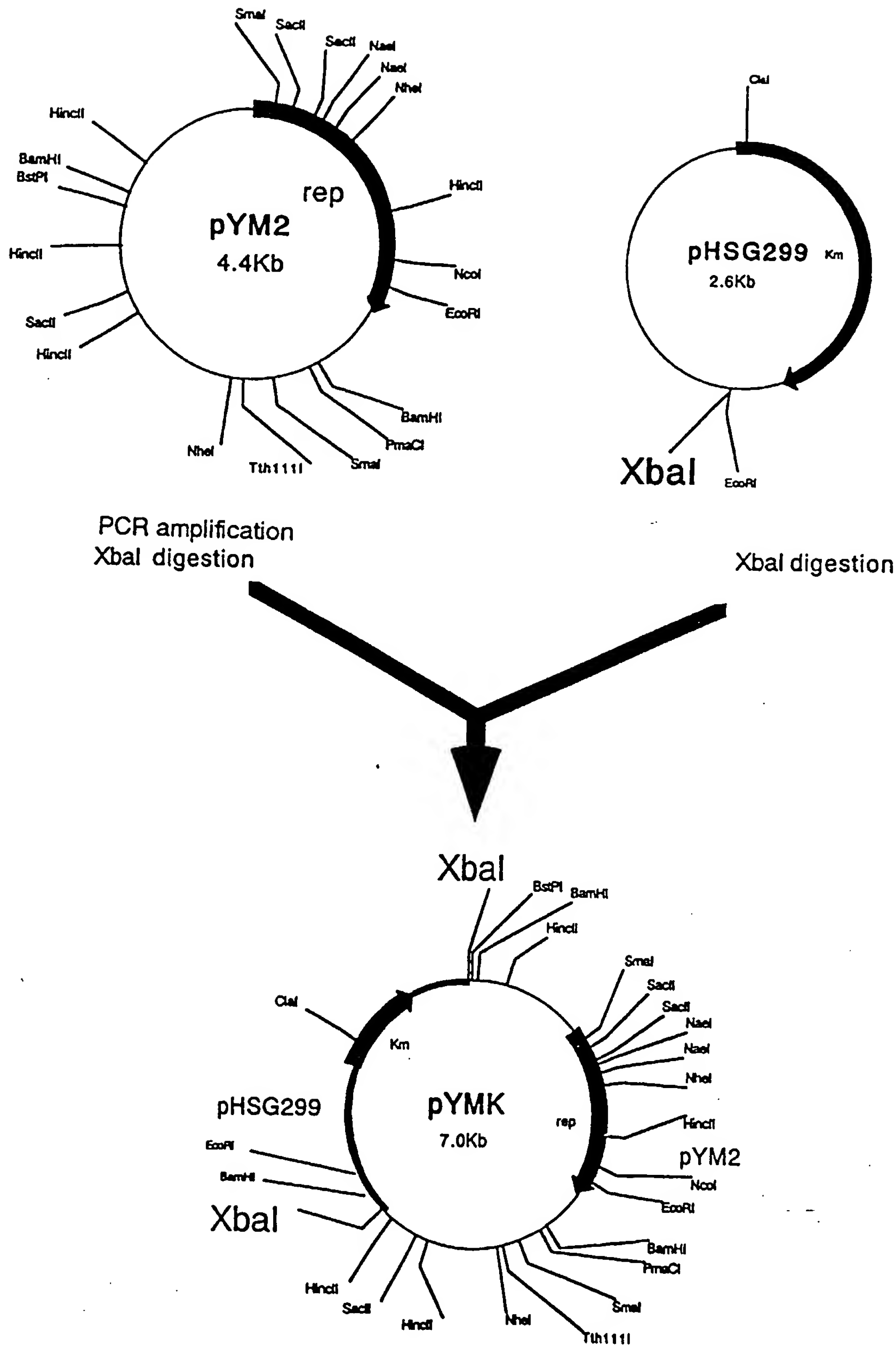
Tth111I

Fig. 3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NcoI
EcoRI
SacII
HincII
BamHI
PmaCI
NheI
Tth111I
SmaI
ClaI
pHSG299
Km
2.6Kb
XbaI
EcoRI
PCR amplification
XbaI digestion
XbaI digestion
XbaI
BstPI
BamHI
HincII
SmaI
SacII
SacII
NaeI
NaeI
NheI
ClaI
Km
pHSG299
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7.0Kb
rep
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NcoI
EcoRI
EcoRI
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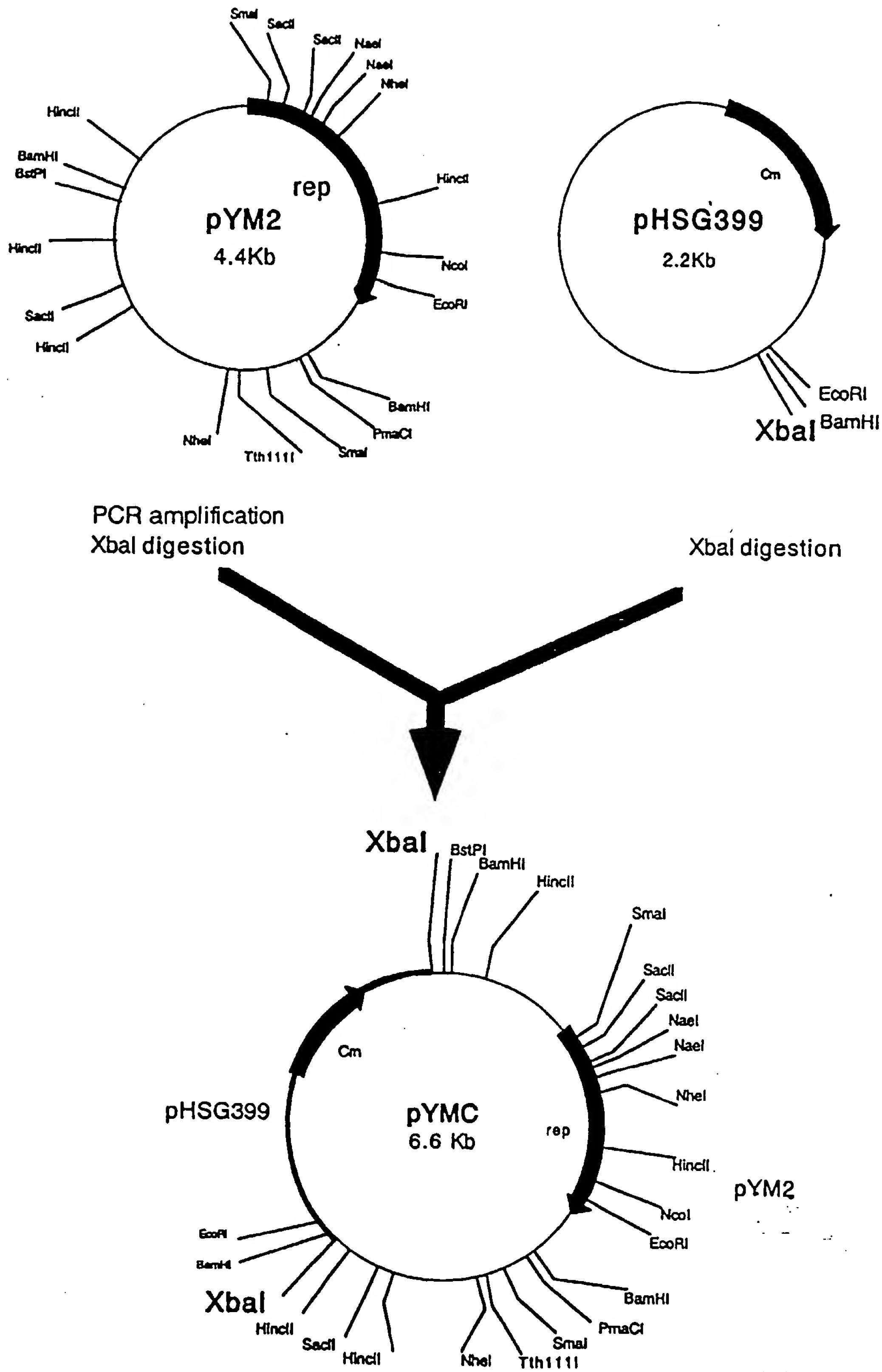
NheI
SmaI
Tth111I

Fig. 4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NcoI
EcoRI
HincII
SacII
HincII
NheI
Tth111I
SmaI
PmaCI
BamHI
Cm
pHSG399
2.2Kb
EcoRI
XbaI
BamHI
PCR amplification
XbaI digestion
XbaI digestion
XbaI
BstPI
BamHI
HincII
SmaI
SacII
SacII
NaeI
NaeI
NheI
Cm
pHSG399
pYMC
6.6 Kb
rep
HincII
pYM2
NcoI
EcoRI
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SacII
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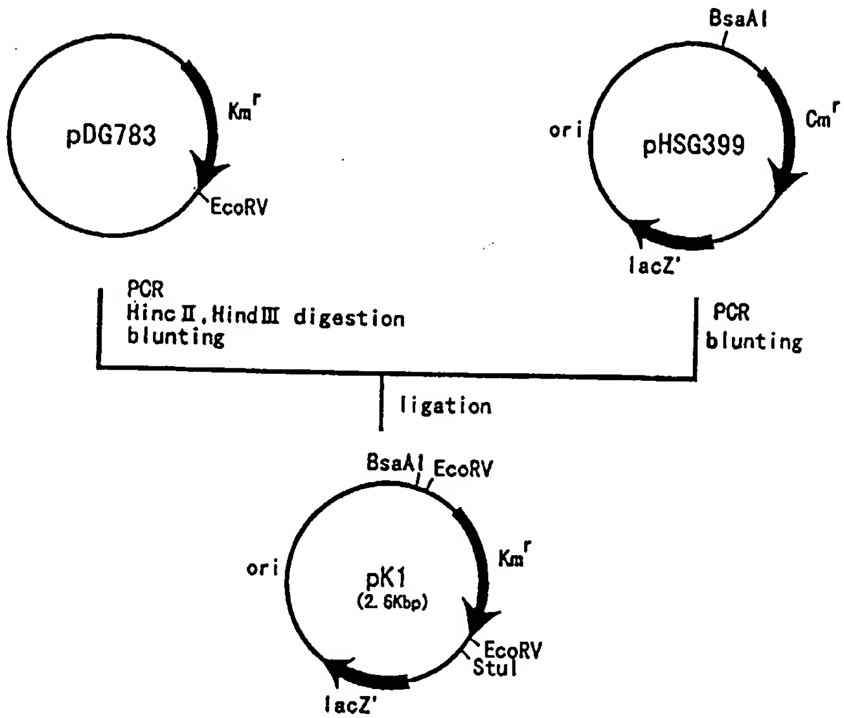
NheI
Tth111I
SmaI
PmaCI
BamHI

Fig. 5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EcoRV
BsaAI
ori
pHSG399
Cm^r
lacZ'
PCR
HincII, HindIII digestion
blunting
PCR
blunting
ligation
BsaAI
EcoRV
ori
pK1
(2.6Kbp)
Km^r
EcoRV
StuI
lacZ'

Fig. 6

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Office européen des brevets

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(71) Applicant: Ajinomoto Co., Inc.
Tokyo 104 (JP)

(72) Inventors:
- Matsuzaki, Yumi, c/o Ajinimoto Co., Inc. Kawasaki-shi, Kanagawa (JP)
- Kimura, Eiichiro, c/o Ajinimoto Co., Inc. Kawasaki-shi, Kanagawa (JP)
- Nakamatsu, Tsuyoshi, c/o Ajinimoto Co., Inc. Kawasaki-shi, Kanagawa (JP)
- Kurahashi, Osamu, c/o Ajinimoto Co., Inc. Kawasaki-shi, Kanagawa (JP)
- Kawahara, Yoshio, c/o Ajinimoto Co., Inc. Kawasaki-shi, Kanagawa (JP)
- Sugimoto, Shinichi, c/o Ajinimoto Co., Inc. Kawasaki-shi, Kanagawa (JP)

(74) Representative: HOFFMANN - EITLE
Patent- und Rechtsanwälte
Arabellastrasse 4
81925 München (DE)

(54) Plasmid capable of autonomous replication in coryneform bacteria

(57) Plasmid isolated from *Corynebacterium thermoaminogenes* or a derivative therof, wherein said plasmid has a size of about 4.4kb or about 6kb and comprises a gene coding for a Rep protein having the amino acid sequence shown in SEQ ID NO: 2 or a sequence at least 90% homologous to the same.

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EUROPEAN SEARCH REPORT

Application Number
EP 00 11 7225

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
A	FR 2 612 937 A (AJINOMOTO KK) 30 September 1988 (1988-09-30) * claims 1,2 * ---	1-5	C12N15/74
A	EP 0 082 485 A (KYOWA HAKKO KOGYO KK) 29 June 1983 (1983-06-29) * page 6 - page 7, paragraph 2 * * examples 1.1,2 * ---	1-5	
A	EP 0 472 869 A (DEGUSSA) 4 March 1992 (1992-03-04) * page 5, paragraph 3 - last paragraph * -----	1-5	
			TECHNICAL FIELDS SEARCHED (Int.Cl.7)
			C12N

The present search report has been drawn up for all claims

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THE HAGUE	16 February 2001	Mata Vicente, T.

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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
FR 2612937 A	30-09-1988	JP 2027856 C	26-02-1996
		JP 7063383 B	12-07-1995
		JP 63240779 A	06-10-1988
		AU 616168 B	24-10-1991
		AU 1161488 A	29-09-1988
		BR 8801289 A	25-10-1988
		KR 9606580 B	20-05-1996
		PH 25252 A	27-03-1991
		US 5250434 A	05-10-1993
EP 0082485 A	29-06-1983	JP 1960412 C	10-08-1995
		JP 6091827 B	16-11-1994
		JP 58105999 A	24-06-1983
		AT 37198 T	15-09-1988
		AU 556761 B	20-11-1986
		AU 9162982 A	23-06-1983
		CA 1199594 A	21-01-1986
		DE 3279030 D	20-10-1988
		DE 82485 T	22-12-1983
		ES 518348 D	16-04-1984
		ES 8404408 A	16-07-1984
		IL 67510 A	31-08-1988
		US 4710471 A	01-12-1987
EP 0472869 A	04-03-1992	DE 4027453 A	05-03-1992
		DE 59100582 D	16-12-1993
		DK 472869 T	03-01-1994
		JP 2603011 B	23-04-1997
		JP 4229183 A	18-08-1992
		US 5175108 A	29-12-1992

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